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Mass-Rearing *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock for the Management of *Hydrilla verticillata*

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PURPOSE: This technical note summarizes the development and application of mass-rearing techniques using ponds at the U.S. Army Engineer Research and Development Center (ERDC) Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX for the biological control agents *Hydrellia pakistanae* Deonier and *H. balciunasi* Bock for use on *Hydrilla verticillata* (L.f.) Royle. Information is provided on rearing methods, releases, and associated costs.

BACKGROUND: Hydrilla, family Hydrocharitaceae, is an exotic submersed macrophyte that is a major problem in the United States (Sutton and Portier 1985). Its propensity for dense growth and ability to reproduce vegetatively interferes with navigation, fishing, boating, and recreational activities (Baloch et al. 1980), and has led to its nickname as the "perfect aquatic weed" by Langeland (1996). Hydrilla possesses physiological characteristics and reproductive strategies that allow for rapid growth and expansion in freshwater environments. Such growth characteristics lead to displacement of native aquatic plant communities and adversely impact freshwater habitats. However, these same characteristics also provide a rapidly replenishing and abundant food supply for exploitation by host-specific herbivores.

Beginning in 1987, two host-specific leaf-mining flies (Diptera: Ephydridae) were released in the United States for the biological control of hydrilla (Buckingham and Grodowitz 2004). Both species are in the genus *Hydrellia* and include *H. pakistanae* originating from the Indonesian region and *H. balciunasi* from Australia (Grodowitz et al. 1994). Numerous studies have examined the effect of *Hydrellia* spp. establishment on hydrilla and have indicated that sustained feeding reduces hydrilla biomass and negatively impacts tuber and turion production, as well as fragment viability (Doyle et al. 2002, 2005; Grodowitz et al. 2003; Owens et al. 2006, 2008).

Hydrellia pakistanae and *H. balciunasi* have been reared under laboratory and greenhouse conditions at the U.S. Army Engineer Research and Development Center (ERDC) since the early 1990's (Freedman et al. 2001). However, this type of rearing is time-consuming, labor-intensive, and expensive (approximately $0.50 per fly). Additionally, the large quantities of healthy plant material necessary to maintain high-producing and high-quality *Hydrellia* spp. colonies are difficult to obtain under such conditions. On the other hand, mass-rearing in ponds is typically easier to maintain and provides the quantities of hydrilla needed. Previously at the LAERF, pond collection has produced a cost estimate of $0.023 per fly (Freedman et al. 2001).

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Pond rearing of *H. pakistanae* has previously been successful at the Tennessee Valley Authority (TVA) pond facility in Muscle Shoals, Alabama (Grodowitz and Snoddy 1995). This facility included earthen, wooden-sided, and greenhouse-enclosed ponds. Populations of *H. pakistanae* became permanently established and self-sustaining at this facility. From 1992 to 1993, over 2,000,000 immature *H. pakistanae* were collected and introduced into hydrilla infestations across the country. Unfortunately, with shifts in research directions within TVA, this facility was closed in 1995 and the ponds allowed to dry, effectively eliminating its rearing capability.

Mass Rearing: The ability to mass-produce large numbers of high quality insect biocontrol agents can be a tremendous asset when implementing a biocontrol program. Common sense would dictate that releasing a high number of agents allows for higher establishment success, more rapid population development, a larger gene pool to minimize genetic drift, and allows for variability in the population to survive changing environmental conditions.

Many challenges are encountered in a rearing program – foremost includes the production of large numbers of high-quality insect agents at reasonable costs. In most cases, laboratory and/or greenhouse-based rearing is highly labor intensive and thereby expensive, and often severe space limitations can curtail production of large numbers. In addition, there is a tendency when using such rearing methods to produce insects with genetic characteristics that may make them unsuitable for survival under field conditions (McKibben et al. 1988, Grodowitz et al. 1992, Center et al.1997). More field-based, mass-rearing methods are needed.

The major objective of this report is to describe the rearing methods employed at the LAERF for *Hydrellia pakistanae* and *H. balciunasi*. Both species have been present in LAERF ponds since 1999, with an unknown method of introduction. The rearing process will be discussed including pond maintenance, quality assessment, population estimation, release and subsequent site monitoring, and overall costs. Criteria used to assess quality of insects include observing establishment success, population rate increase, and damage to hydrilla, both at field-release sites and in rearing ponds.

MATERIALS AND METHODS

Pond Maintenance. A variety of procedures were used to maximize hydrilla production in the ponds and maintain a surface canopy for a majority of the growing season. Surface canopy is important because it provides an ovipositioning substrate for the female *Hydrellia* spp. These manipulations were accomplished in an effort to maintain healthy hydrilla throughout the growing season, providing for an increasing and sustained fly population. Initially, ponds previously containing hydrilla were drained and allowed to remain dry over the entire winter. In late spring or early summer, the ponds were tilled to disturb the existing hydrilla tuber bank, thereby inducing and maximizing sprouting. In addition, water was added to the ponds in small increments to allow maximum light penetration throughout the initial hydrilla growth period. Matching water level to hydrilla growth allowed for maximum hydrilla growth while maintaining a surface canopy.

Mass-Rearing/Release. Fly numbers were monitored approximately monthly by collecting 10 hydrilla stems at random, ranging in length from 10 to 25 cm, from each rearing pond and enumerating immatures (i.e., three larval instars and pupal stage). Length and fresh weight of each stem was recorded, as well as number of immature flies in each stage of development. The number

of immatures in each stem was divided by the fresh weight of each stem, providing number of flies per hydrilla fresh weight.

In 2005 in Texas, 17 potential release sites were visited. Field assessments of potential release sites were conducted in coordination with the local managing authority of each water body. In conducting an assessment, the relative amount of topped-out, contiguous hydrilla was noted, as was the location within the reservoir (cove, boat lane, etc.). Sites containing large contiguous mats of hydrilla provided ample material for introducing *Hydrellia* spp. and assuring high establishment success. Preference was made for releases in coves as opposed to boat lanes, as boat traffic might disturb the mat and prevent the colonizing flies from spreading rapidly. Hydrilla samples were collected at each site within the lake to determine fly establishment. From these, fly larvae were enumerated by microscopic examination as well as Berlese extraction with the same methods previously mentioned to monitor rearing pond numbers. Generally, two or three sites per reservoir were chosen for fly release.

Once it was determined that a particular reservoir offered high-quality sites for release, the controlling authority was contacted and *Hydrellia* spp. were harvested from the LAERF for shipment. Fly-infested hydrilla was collected from ponds with the largest observed numbers per unit weight to reduce shipping costs. Hence, number of flies on a per weight basis was used in calculating approximate number of flies shipped for release. The infested material was packaged and transported in insulated ice coolers for introduction. Total weight of shipped hydrilla was used to estimate fly numbers shipped. The reservoir controlling authorities were provided instructions on site locations and methods of release. Instructions included spreading the hydrilla fragments over the surface of the mat, making sure the fragments were below the water surface to prevent desiccation.

Costs. Total costs for mass rearing were determined and included those associated with both rearing and field release. Costs included man-hours involved in pond maintenance (e.g., draining, tilling, refilling, mowing around the edge of pond), insect enumeration, and insect collection for shipment. Shipping supplies were also included in the total cost (ice coolers, packing tape, and plastic trash-can liners). Shipping costs were estimated using approximate weight of full shipping containers, the “Priority Overnight” Fedex™ shipping option, and actual origin and destination ZIP codes.

RESULTS/DISCUSSION

Pond Maintenance. Tilling the dry ponds was apparently a viable option aiding in rapidly establishing a hydrilla infestation. Observations indicated rapid regrowth in early spring soon after tilling the ponds and adding water. Hydrilla growth from tubers was adequate and tilling apparently decreased the amount of other macrophytes present that could compete with hydrilla, thereby reducing overall biomass, surface coverage, and quality.

While it was relatively easy to maintain water level in the ponds to match and maximize hydrilla growth, it is unknown whether this was a viable option, since no experimental trials were conducted comparing differences with and without water level fluctuations. However, common sense would dictate that the more light available to the plants, especially in early growth stages, the faster growing and more robust the plants should be. More research is warranted.

Mass-Rearing. Pond manipulations at the LAERF to produce vigorous hydrilla were successful in maximizing numbers of flies produced. For example, number of *Hydrellia* spp. shipped from LAERF has increased approximately 32-fold in just five years; i.e., from 2000 to 2005 (Figure 1) as a result of better pond maintenance techniques, population increases, and increased knowledge of fly availability at the LAERF. Approximately 12,700,000 immature *Hydrellia* spp. flies were released from August 2005 to November 2005, with each release site receiving approximately 1,000,000 immature *Hydrellia* spp. This is six-fold greater than previous field rearing at the TVA; in 1992-1993, more than 2 million *H. pakistanae* were released at field locations (Grodowitz and Snoddy 1995).

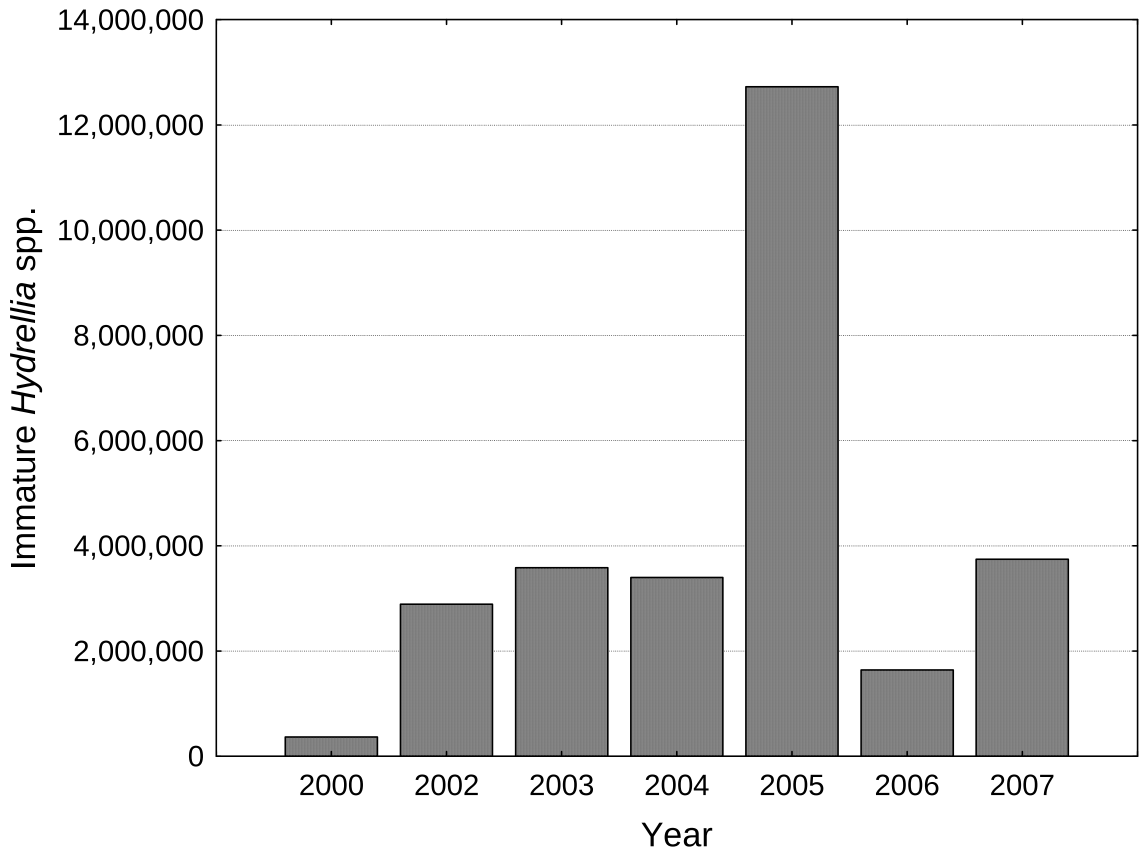

Figure 1. Number of *Hydrellia* spp. immatures shipped from 2000 to 2007.

From 2000 to 2008, over 28 million immature flies have been released from the LAERF at over 30 sites in six states including Texas, Florida, North Carolina, Virginia, Georgia, and Arkansas. This can be attributed to several factors. First, rearing efficiency and methods have improved such that less effort can produce the same numbers of flies. It is now possible to release more control agents at less cost. While populations have steadily increased, budget cutbacks have reduced the number of releases since 2005. Despite the lack of releases in the last few years, as *Hydrellia* spp. have become established and further damage has been observed in hydrilla-infested water bodies, the demand for the flies has increased.

Another technique to maximize number of flies harvested involved estimating fly populations throughout the growing season, allowing fly collections and shipment to proceed at times of greatest fly density. Based on records collected over four years, fly numbers are typically highest in late summer/early fall (Figure 2). Understanding what time of year the fly populations are greatest in the ponds aids in shipping efficiency; i.e., most immatures, least amount of work and plant matter, etc. Costs are also decreased with establishment and steady population growth, which also increases efficiency (See section titled “Costs” below). In the initial stages of a rearing program, costs will decrease as populations increase to a maximum.

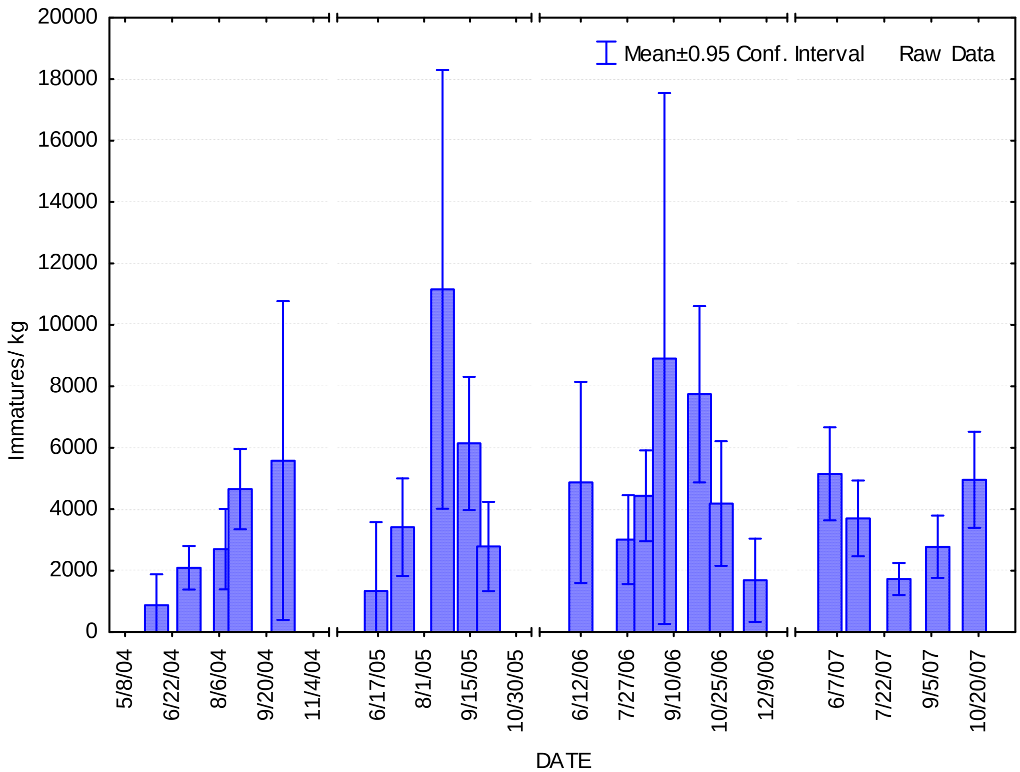

Figure 2. Mean *Hydrellia* spp. immatures per kg fresh weight of *H. verticillata* in rearing ponds at the LAERF from June 2004 to October 2007.

In 2001, *H. pakistanae* and *H. balciunasi* were present in ponds at a ratio of 1.57:1 (unpublished data). Currently, *H. balciunasi* populations have decreased to minimal numbers, so it is likely the vast majority of agents released have been *H. pakistanae*.

Field rearing of *H. pakistanae* has several advantages over traditional laboratory rearing. Once established, a self-sustaining steady source of agents is available for shipment. The amount of labor involved in field rearing is also substantially less than in a laboratory. With an ample supply of healthy host plants, the agent population can steadily increase over time.

Costs. Cost analyses were performed for *Hydrellia* spp. rearing during 2005 at the LAERF. Other years were not considered because insufficient data exist regarding shipping costs, man-hours, etc. Shipping the infested plant material in large ice coolers, with the “Priority Overnight” option (Figure 3) accounted for 77 percent of costs incurred ($17,094 out of $22,119). *Hydrellia* spp. costs

are estimated at $0.0018/fly. This is a substantial decrease from the previously estimated $0.023/fly in field rearing at the LAERF (Freedman et al. 2001). This is also considerably less cost than $0.50/fly reared in the greenhouse facility at ERDC.

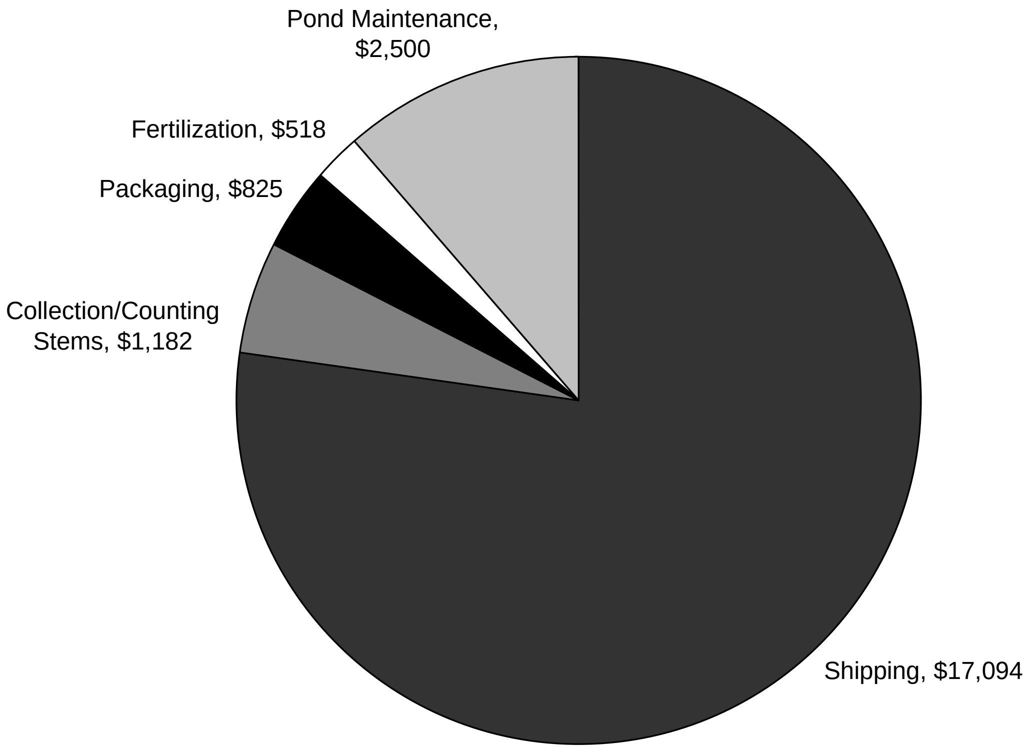

Figure 3. Cost breakdown of *Hydrellia spp*. rearing at the LAERF during the 2005 season, in which approximately 12 million flies were released.

As previously stated, the data indicate that as pond *Hydrellia* spp. population densities increase over time, costs associated with delivery and subsequent release will decrease (Figure 4). For example, if population estimates determine a pond density of 1500 immatures per kilogram hydrilla fresh weight, and the cost to ship one cooler containing 15.8 kg of hydrilla from Lewisville, TX to Okahumpka Lake, FL is approximately $194.88, then the shipping cost per fly is $.0082 (Table 1). When *H. pakistanae* population density reaches 5,000 immatures per kilogram hydrilla fresh weight, the cost per fly decreases to $0.0025. This general relationship can be seen in Figure 4. This is important to the rearing process in that with diminishing returns, similar gains can be achieved earlier in the season. Because of this, flies can be released earlier in the year at a similar cost. This allows the flies more time to establish and build field populations before winter.

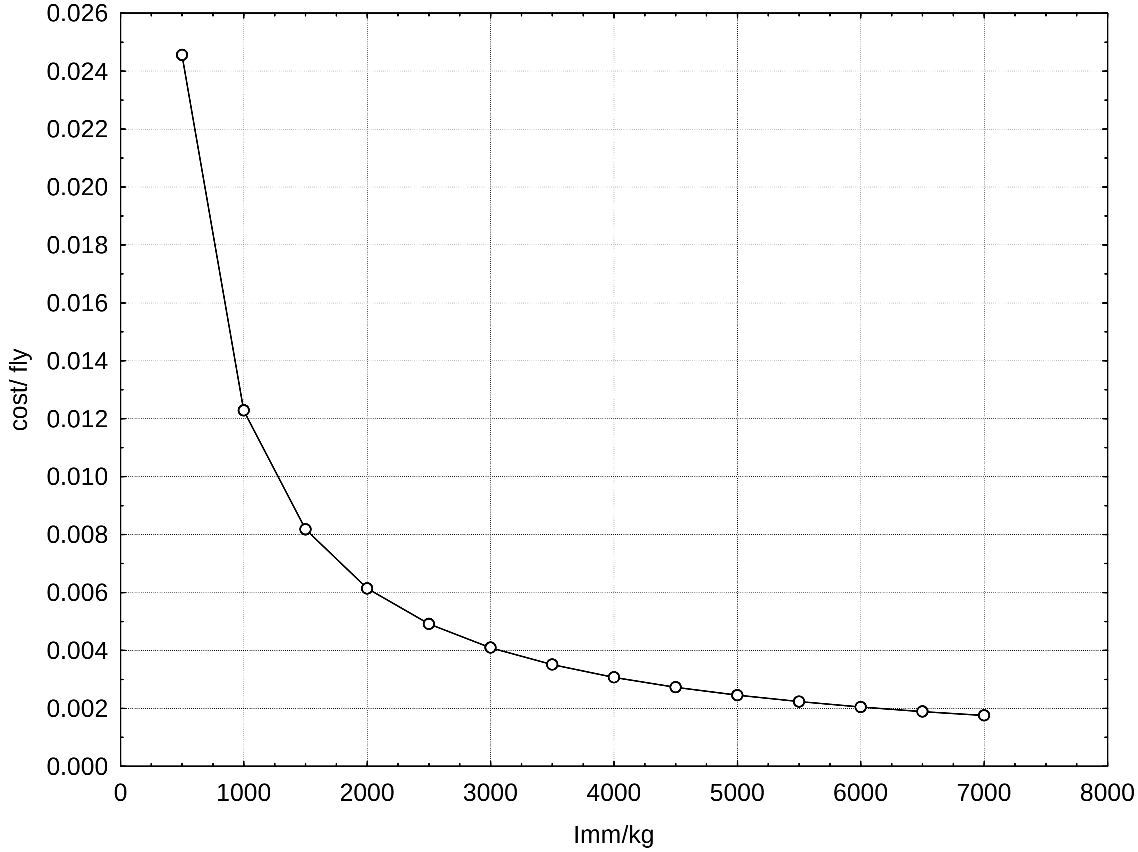

Figure 4. Hypothetical estimation of cost per fly with varying densities of biocontrol agent (costs estimated using a constant weight).

Table 1. Hypothetical calculation of *Hydrellia pakistanae* production cost estimates using low (1,500 Imm/kg) and high (5,000 Imm/kg) fly densities.

Mean Pond Density	Fresh Weight Hydrilla	Number of Flies Shipped for Release	Cost of Overnight Shipping (Fedex® from Lewisville, TX to Lake Okahumpka, FL)	Cost per fly
1,500 Imm/kg	15.875 kg	23,812	$194.88	$0.0082
5,000 Imm/kg	15.875 kg	79,375	$194.88	$0.0025

Establishment. Establishment success is an important criterion in evaluating fly quality. Toward this goal, *Hydrellia spp*. were released at several lakes around Texas in the years prior to 2005. In summer 2005, many of these lakes were visited to monitor the status of *Hydrellia* spp. establishment. Both previous release sites and potential release sites were visited. Establishment success has been high with at least 78 percent of release attempts having *Hydrellia* spp. present 10-12 months or longer after initial releases. This is an increase from the 55 percent establishment reported previously from laboratory-reared insects (Freedman et al. 2001). Center et al. (1997) reported more than 70 percent establishment success with *H. pakistanae* only after modifying their rearing methods, including the release of individuals that had not been through a long-term laboratory rearing program.

The most impressive of the previous release sites in Texas is Sheldon reservoir (Figure 5), in which the mean immatures per kilogram was recorded as 549 in 2005. In the first year of site examination (1998) at Sheldon reservoir, fly numbers were not detectable. By 2002, flies had been detected in the reservoir and were estimated at 262 immatures per kilogram. These were then supplemented with a release in 2004 leading to the 2005 population estimate. As early as 2004, the hydrilla had begun to appear unhealthy (brown stems and leaves). A year later, *Hydrellia* spp. larvae were observed in hydrilla leaves at every site visited and 100 percent damage to topped-out plants was common. By 2006, less than 20 strands of hydrilla were found during the yearly visit. Those that were found were 1-2 ft below the water surface.

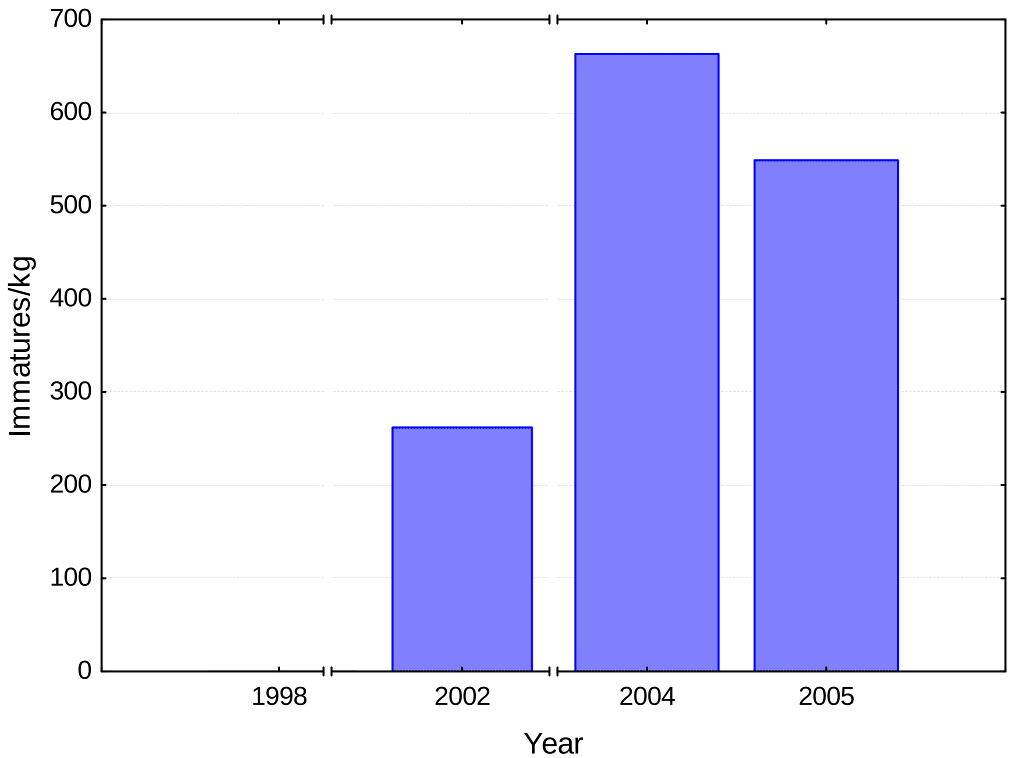

Figure 5. Mean fly populations in Sheldon reservoir, TX since 1998. Sites were not visited in the years 1999-2001.

Furthermore, at 68 percent of release sites, decreases in hydrilla and/or increases in natives have been noted 2-3 years after fly introduction. Although other environmental factors, such as nutrient depletion, water level changes, turbidity, etc., could have influenced the hydrilla declines, feeding damage by *Hydrellia* spp. flies apparently weakened and stressed the hydrilla aiding in the decreases. With the increasing number of controlled experiments showing fly impact to biomass, tubers, and turions (Owens et al. 2006, 2008), and the ability to rear high numbers of flies inexpensively, it is hard to dismiss the importance of this biocontrol agent for hydrilla management.

FUTURE PLANS: Scientists at the LAERF are currently experimenting with seasonality and fertilization of hydrilla to increase *Hydrellia spp*. production by maintaining a continuous supply of healthy plants for the flies' food source. It is hoped that drying ponds periodically, tilling the sediment, and refilling at various times of the year will provide new plants for the flies over the

entire growing season. All of these areas require further investigation in order to determine their effectiveness.

In addition to nutritional supplementation, an area of potential research lies in altering water levels to maintain topped-out hydrilla. Because the adult *Hydrellia* spp. requires surface vegetation for oviposition, raising water levels after hydrilla has reached the surface may be detrimental to population growth. The cost/benefit ratio needs to be analyzed.

Overwintering behavior of *Hydrellia pakistanae* is currently under examination at the LAERF. Results of this study will aid in winter pond maintenance and help quantify population numbers in months when adults and larvae decrease to minimal numbers.

POINTS OF CONTACT: This technical note was written by Nathan Harms, University of North Texas – Lewisville Aquatic Ecosystem Research Facility, and Michael Grodowitz and Julie Nachtrieb at the U.S. Army Engineer Research and Development Center, Vicksburg, MS. For additional information, contact the acting manager of the Aquatic Plant Control Research Program, Dr. Linda Nelson (601-634-2656, *Linda.S.Nelson@usace.army.mil*) or Dr. Al Cofrancesco, Technical Director, Civil Works Environmental Engineering and Science (601-634-3182, *Al.F.Cofrancesco@usace.army.mil*). This technical note should be cited as follows:

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